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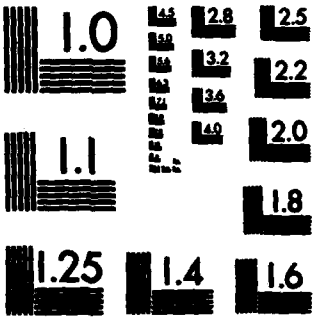

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

8-mercaptoguanosine (8sGuo) is an adjuvant that can be used to enhance the in vivo response of mice to polysaccharide antigens. This enhancement of the immune response is observed in all of the IgG subclasses examined, IgG_1, IgG_2, and IgG_3. Most interestingly injection of this adjuvant restores the responses of nonresponder immune defective CBA/N mice to TNP-Ficoll the prototype

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polysaccharide antigen we use in these experiments. Concomitant administration of this adjuvant together with TNP-Ficoll also enhanced the antibody responses of low responder neonatal mice to this antigen. Since our results suggested that this immune adjuvant was being rapidly metabolized *in vivo* we determined whether continuous injection of this adjuvant was more effective than a single dose. Our results indicate that injection of 8sGuo on three consecutive days was significantly more effective in enhancing anti-TNP-Ficoll responses than administration on one day only. We have determined from *in vitro* experiments that 8sGuo is exerting its predominant effect on activated and not resting B cells. Taken together our data suggest that this immune adjuvant will serve as a useful tool to investigate the regulatory controls of immune responses to polysaccharide antigens in normal and immune defective mice as well as in neonatal mice.

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I. Enhancement of In Vivo Responses to Polysaccharide antigens with 8-mercaptoguanosine (8sGuo)

A. Responses to trinitrophenylated AECM-Ficoll. In view of the published reports by Goodman and Weigle demonstrating enhancement of antibody responses to TNP-Ficoll *in vitro* using 8sGuo we initiated a series of experiments to determine the effects of this adjuvant *in vivo*. When antigen and adjuvant are injected simultaneously there is on average a 10-100 fold increase in the serum IgG_2 anti-TNP titer seven days later (Table I).

Table I. 8sGuo enhances the *in vivo* antibody response to TNP-Ficoll.

	IgG_1	IgG_2	IgG_3	IgM
TNP-Ficoll	160 (1.38)	58 (1.49)	18 (1.37)	260 (1.19)
TNP-Ficoll +8sGuo	975 (1.34)	831 (1.29)	142 (1.60)	283 (1.15)

The enhancement seen in the IgG_1 and IgG_3 antibody subclasses was not as dramatic as that seen in the IgG_2 subclass but was significant and reproducible. The peak antibody response was always noted seven to ten days after immunization and by day fourteen was declining (Table II).

Table II. Kinetics of the 8sGuo induced enhancement of the antibody response to TNP-Ficoll.[1]

	Day 7	Day 10	Day 14
		IgG_2 anti-TNP titer	
TNP-Ficoll	180 (1.68)	124 (1.13)	117 (1.32)
TNP-Ficoll + 8sGuo	1,108 (1.21)	517 (1.60)	379 (1.47)

1. Groups of 6 mice were injected with TNP-Ficoll (10 μg) and 8sGuo and sera obtained 7, 10 and 14 days later. Results represent geometric mean ($\bar{x}$/ ÷log s.e.).

Whether the antigen was administered by intravenous or intraperitoneal route appeared to have little effect on the ability of 8sGuo to effect an enhanced response, however the enhancement was very dependent on the amount of 8sGuo injected (Table III).

Table III. 8sGuo induced enhancement of the IgG_2 anti-TNP antibody response to TNP-Ficoll requires a high dose of adjuvant.[1]

	Dose of 8sGuo	Day 7	Day 10
		IgG_2 anti-TNP-titer	
TNP-Ficoll	-	42 (1.38)	368 (1.23)
TNP-Ficoll	30 mg	444 (1.27)	409 (1.23)
	10 mg	81 (1.39)	58 (1.23)
	3 mg	68 (1.19)	-

1. Group of 6 CBA/J mice were injected with TNP-Ficoll (10 μg) together with 30 mg, 10 mg or 3 mg of 8sGuo. Sera was obtained 7 and 10 days later.

Thus injection of less than 30 mg of 8sGuo per mouse was only marginally effective in enhancing the anti-TNP-Ficoll response, while doses of 50 - 100 mg per mouse were no more effective than 30 mg. This observation suggested to us the possibility that the adjuvant may be rapidly consumed *in vivo* and therefore large doses and possibly continuous administration of the adjuvant may be the most effective way to elicit an enhanced response. To test this we injected TNP-Ficoll on day 0 and 8sGuo on five consecutive days thereafter and compared its effect to that seen when the adjuvant is given on one day only (Table IV).

Table IV. Repeated injection of 8sGuo is more effective than a single injection in enhancing the anti-TNP-Ficoll response.

	Anti-TNP-Titer IgG_1	Anti-TNP-Titer IgG_2
TNP-Ficoll	863 (1.26)	141 (1.08)
TNP-Ficoll + 8sGuo (Day 0)	5,163 (1.05)	2,670 (1.14)
TNP-Ficoll + 8sGuo (Day 0 - Day 5)	19,229 (1.11)	27,521 (1.13)

1. Groups of 6 mice were injected with 10μg of TNP-Ficoll only, or together with 8sGuo or were given 8sGuo on days 0 through day 5. Sera were obtained seven days later and titered for the presence of anti-TNP-antibody.

From other data it appears that administration of the adjuvant on day 0 - day 3 was also significantly more effective in enhancing the response to TNP-Ficoll than when it was given on one day only.

B. Responses to pneumococcal polysaccharide type 14 (P-14). Having established that responses to a haptenated-polysucrose molecule (i.e.; TNP-Ficoll) could be readily enhanced by 8sGuo, we attempted to test another purified polysaccharide antigen (P-14) (Table V).

Table V. Enhancement of the IgG_1 and IgG_2 antibody responses to pneumococcal polysaccharide type 14 by 8sGuo.[1]

	anti-P-14-titer IgG_1	anti-P-14-titer IgG_2
P-14	<10	24 (1.24)
P-14 + 8MG	160 (1.40)	296 (1.30)

1. Groups of 6 mice were injected with 20 μg of pneumococcal polysaccharide type 14 (P-14) and sera obtained seven days later.

Responses to this antigen in the absence of adjuvant were very low and often below our level of detectability. In the presence of 8sGuo, however, this antigen stimulated a significant response which was always greater than that seen in the absence of adjuvant. Although the maximum

enhancement was seen when antigen and adjuvant were given together, significant enhancement of the response was observed even when antigen was injected seven days prior to the adjuvant (Table VI).

Table VI. 8sGuo enhances IgG_2 anti-P-14 response even when injected seven days after injection of the antigen.

		IgG_2 anti-P-14 titer
P14 (day - 7)		39 (1.46)
P14 (day - 7)	+8sGuo (day 0)	148 (1.40)
P14 (day 0)		37 (1.50)
P14 (day 0)	+8sGuo (day 0)	622 (1.45)

1. Groups of 6 mice were injected with 20 μg of P14 either 7 days prior to injection of 8sGuo (day - 7) or simultaneously with 8sGuo (day 0). Sera was obtained seven days after injection of 8sGuo.

Similar degrees of enhancement were observed when a mixture of pneumococcal polysaccharides (Pneumovax) were injected (data not shown).

II. Elicitation of in vivo responses to TNP-Ficoll in xid immune defective CBA/N mice.

CBA/N mice carry an x-linked immune defect which precludes them from responding to polysaccharide antigens. In this regard they resemble the immune system of newborn mice which are also hyporesponsive to this group of antigens. To test whether 8sGuo could convert a "non-responder" strain into a "responder" strain, we immunized CBA/N mice with TNP-Ficoll and 8sGuo. We observed that administration of 8sGuo on five consecutive days to these mice restored the anti-TNP-Ficoll responses to a level which was comparable to that seen in control responder mice (Table VII).

Table VII. CBA/N mice with the xid immune defect respond to TNP-Ficoll in the presence of 8sGuo.

	Anti-TNP-Titer			
	IgG_1		IgG_2	
	Day 10	Day 14	Day 10	Day 14
TNP-Ficoll	<5	<5	<5	<5
TNP-Ficoll +8sGuo	361 (1.07)	680 (1.15)	1,272 (1.10)	467 (1.13)

1. Groups of 6 CBA/N x DBA/2 male mice were injected with TNP-Ficoll (10 μg) on day 0 and with 8sGuo on day 0 through day 5. Results represent geometric mean (x/÷log s.e.).

This enhancement of responsiveness was seen in the IgG_1 and IgG_2 subclasses but not in the IgM and IgG_3 subclasses of antibody.

III. In vitro enhancement of polysaccharide responses by 8sGuo

A. Lack of requirement for continuous antigen. We wished to determine whether the 8sGuo induced enhancement of in vivo responses to TNP-Ficoll (Table VIII) was dependent on the continued presence of the antigen.

Table VIII. Enhancement of anti-TNP PFC response does not require the continuous presence of antigen.

"Priming" Antigen	"Primed" B Cells Cultured With: Medium			8sGuo (0.25mM)		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
Medium	5(1.22)	12(1.95)	12(1.18)	30(1.15)	120(1.30)	130(1.01)
TNP-Ficoll 2.5ng/ml	17(1.54)	13(1.08)	9(2.34)	293(1.12)	598(1.07)	320(1.18)

1. B cells from CBA/J were cultured for 48 hours at 5 x 10^5 cells/ml with medium only or with TNP-Ficoll (2.5ng/ml) and then washed 3 times with Mishell Dutton medium and placed into culture with medium, 8-Mercaptoguanosine (0.25mM) or with 8-Mercaptoguanosine + TNP-Ficoll (2.5ng/ml) and then assayed for anti-TNP PFC 24, 48, or 72 hours later.
2. Results represent geometric mean (x/÷ log s.e.) of triplicate wells.

was dependent on the continued presence of the antigen. To this end purified populations of murine B cells were cultured with TNP-Ficoll for 48 hours and then extensively washed. 8sGuo was added for an additional 48 hours and numbers of cells secreting anti-TNP antibody were enumerated. The number of anti-TNP PFC counted was equivalent to that seen when antigen and 8sGuo were cultured for the duration of the four day culture period. This suggested that the 8sGuo induced enhancement of the response to TNP-Ficoll did not require the continued presence of high concentrations of this antigen.

B. Rapid onset of action of 8sGuo. Since it was clear from our results as well as from those published reports of Goodman and Weigle that 8sGuo did not have to be added at the onset of culture, we wished to determine the minimum time that 8sGuo needed to be cultured with cells to exert its maximum effect (Fig. 1). B cells were cultured with antigen for 48 hours and 8sGuo added 12,24 or 48 hours prior to enumeration of anti-TNP plaque forming cells. Although enhancement of in vitro responses were observed even 12 hours after the addition of 8sGuo, the maximum enhancement was observed when 8sGuo was added for 48 hours prior to enumeration of antibody secreting cells.

C. 8sGuo exerts its effect on partially activated and not on resting B cells. Although 8sGuo had been shown by Drs. Goodman and Weigle to be an effective polyclonal B cell activator it was not clear whether it was exerting its effect on all the B cells or only a subset of them. To address this issue we separated B cells using cell size as the criterion reflecting the degree of activation of the B cell. We have previously shown that elutriation of B cells was an effective technique that could be used to separate small (115 μ^3) B cells from large B cells (175 μ^3). Interestingly, 8sGuo

stimulated thymidine incorporation only in large B cells and not in small "resting" B cells. Furthermore while this adjuvant could induce a significant increase in the expression of B cell Ia, it was only marginally effective in stimulating such an increase in small resting B cells (data not shown). Thus it appears that 8sGuo is an effective B cell activator but only on B cells that have been previously activated either by intentional administration of antigen or by environmental antigen. Resting B cells in G_o appear to be resistant to the polyclonal B cell enhancing effects of 8sGuo.

D. 8sGuo is an effective adjuvant for neonatal B cells. Having demonstrated that 8sGuo could enhance in vivo responses to TNP-Ficoll even in a non-responder strain of mice (CBA/N) we wished to determine whether responses of neonatal mice to this polysaccharide antigen were also enhanceable. Spleen cells from 3, 6 and 11 day old mice were cultured with TNP-Ficoll and 8sGuo (Table IX) and anti-TNP plaque forming cell responses enumerated 3 days later.

Table IX. 8-Mercaptoguanosine restores in vitro responsiveness of neonatal spleen cells to TNP-Ficoll.

Age of Mice	8-Mercaptoguanosine	TNP-Ficoll	TNP-Ficoll + 8-Mercaptoguanosine
3 days	30 (1.58)	3 (1.80)	114 (1.32)
6 days	76 (1.38)	0	222 (1.14)
11 days	73 (1.43)	3 (1.25)	190 (1.15)

Although B cells from these young mice are unresponsive to this antigen in the absence of 8sGuo they are responsive in its presence. Thus it appears that TNP-Ficoll could initiate the early steps of B cell activation even in immature B cells of neonatal mice and 8sGuo could exert its immuno-enhancing effects on these activated B cells.

Taken together these data provide strong evidence that the immune adjuvant 8sGuo will be useful as an approach to studying ways in which the immune response to even poor immunogens such as polysaccharides can be enhanced. Furthermore it gives one a tool to study how an immature immune system which is unresponsive to polysaccharides can be converted into a responder status.

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